TNF cytokine family: **More BAFF-ling complexities**

Yacine Laâbi, Alexander Egle and Andreas Strasser

Recent studies on BAFF, a member of the tumor necrosis factor family, and the discovery of a new BAFF receptor have revealed that this ligand–receptor pair is essential for B-cell survival and differentiation, holding promise for a better understanding and treatment of some autoimmune diseases and lymphomas.

Address: The Walter and Eliza Hall Institute of Medical Research, PO Royal Melbourne Hospital, 3050 Victoria, Australia.
E-mail: strasser@wehi.edu.au

Current Biology 2001, 11:R1013–R1016

0960-9822/01/$ – see front matter

Members of the tumor necrosis factor (TNF) cytokine family regulate a wide range of biological responses, such as inflammation and apoptosis. The recent discovery of a new member of the TNF family, BAFF, and the identification of its receptors, together with the description of their functions in immune responses, reads like a gripping detective story. It has also sparked a competitive race between biotechnology companies hoping to use this information to design drugs for the treatment of certain autoimmune diseases and lymphoma [1]. BAFF (also called BLys, TALL-1, THANK, zTNF4) was discovered in 1999 as a TNF-related cytokine by sequence-homology-based database searches [2,3]. Two receptors for BAFF were identified soon after [1] but it was clear that there was more to the story. Now a number of papers (including one in this issue of *Current Biology*) describe a new important receptor for BAFF and provide interesting insights into the role of BAFF in B-cell survival [4–7].

BAFF is produced by macrophages, dendritic cells and T lymphocytes. It promotes survival of B cells in culture and increases their proliferation in response to mitogenic stimulation [2,3]. Expression of a BAFF transgene in mice caused accumulation of immature and mature B cells, increased serum immunoglobulin (Ig) levels and elicited systemic lupus erythematosus (SLE)-like autoimmune symptoms [8–10]. BAFF therefore promotes survival and differentiation of autoreactive B cells, which would normally be deleted at the B-cell receptor checkpoint. In contrast, BAFF-deficient mice have a profound block in B-cell development at the B-cell receptor checkpoint [4,11]. These mice have normal numbers of short-lived pro-B, pre-B and immature B cells but failed to accumulate mature B cells (Table 1). Thus, BAFF is essential for the transition through the B-cell receptor checkpoint and for sustained survival of long-lived B cells. BAFF-deficient mice also have profound defects in antibody-mediated immune responses and abnormally low serum Ig levels. It is unclear whether this defect reflects a role for BAFF in B-cell activation or is simply a consequence of the shortage of antigen-reactive B cells in these mice.

APRIL (also called TRDL-1α), another TNF family member found by database mining [12], is the closest known relative of BAFF and is produced by macrophages. APRIL stimulates proliferation of transformed cells and, intriguingly, high levels of APRIL have been detected in many types of cancers. These findings may indicate that APRIL plays a role in tumorigenesis, either as an autocrine growth factor or perhaps to stimulate angiogenesis.

At the time of the discovery of BAFF and APRIL, two members of the TNF receptor family — BCMA and TACI — were recognized as ‘orphan receptors’. Typically TNF receptor family members contain in their extracellular region several modules containing six cysteines that form three disulfide bonds. TACI and BCMA are most closely related to each other, but are unique in that BCMA has only one such module in its ligand-binding region, while TACI has two. Studies with recombinant proteins identified ligands for these receptors and demonstrated that BAFF and APRIL interact with both TACI and BCMA, but not with the other TNF receptor family members known at that time [9,13–15]. These receptor–ligand interactions are of significant affinity, because injection or transgenic expression of soluble forms of the TACI and BCMA receptors achieved effective ligand neutralization that was sufficient to dampen antibody-mediated immune responses ([9,15] and J. Tschopp, personal communication).

It was therefore thought that TACI and BCMA represent essential receptors for BAFF and APRIL. However, there were inklings that matters may not be that simple. For example, BAFF and APRIL could bind to certain cell lines that expressed neither TACI nor BCMA [5,12]. Nevertheless, it came as a surprise that TACI- and BCMA-deficient mice have no major defect in the antigen-independent stages of B-cell development (Table 1). No B-cell abnormalities were found in BCMA-deficient mice [4,16]. Surprisingly, TACI-deficient mice even had mild B-cell hyperplasia [17,18], indicating that TACI may inhibit B-cell production by acting as a decoy receptor for BAFF. Interestingly, TACI-deficient mice had impaired responses to so-called type II T-cell-independent (TI) antigens (prototypically NP-Ficoll) but normal antibody-mediated responses to all other types of antigens. Responses to type I TI antigens, such as lipopolysaccharide, may be independent of TACI, because they, unlike type II TI

Table 1

Immunological phenotypes of selected relevant knockout and transgenic mouse models.

Mouse system	Bone marrow-pro/pre-B cells	Late transitional B cells	Mature B cells	Serum IgG	Serum IgM	TD-I*	TI-I†
baff$^{-/-}$ [4,11]	Normal	–	–	–	–	–	–
"*baff-r*$^{-/-}$" (A/WySnJ) [5]	Normal	n.a.‡	–	–	Normal	–	Normal
taci$^{-/-}$ [17,18]	Normal	+	++	Normal	Abnormal	Normal	–
bcma$^{-/-}$ [4,16]	Normal	n.a	Normal	Normal	Normal	Normal	Normal
taci-Ig tg§ [11,15]	Normal	–	–	–	–	–	–
nik$^{-/-}$ (*aly*-mice) [22]	n.a.	–	–	–	–	–	n.a.
ikk-α$^{-/-}$ [23,24]	Normal	–	–	–	–	–	n.a.
c-rel$^{-/-}$/*rel-a*$^{-/-}$ [25]	n.a.	–	–	n.a.	n.a.	n.a.	n.a.
baff tg [8–10]	Normal	+++	+++	+++	+++	++	+++
bcl-2 tg [19]	Normal	++	++	++	++	++	n.a.
bim$^{-/-}$ [20]	Normal	++	++	++	++	n.a.	n.a.

*TD-I (T-cell-Dependent Immune responses). †TI-I (T-cell-Independent Immune responses; type II). ‡n.a. not analysed, not reported. §tg transgenic.

antigens, can activate Toll-like receptors. Interestingly, Toll-like receptors and TNF receptor family members stimulate some of the same transcription factors, for example Rel/NF-κB, indicating that they may have overlapping functions.

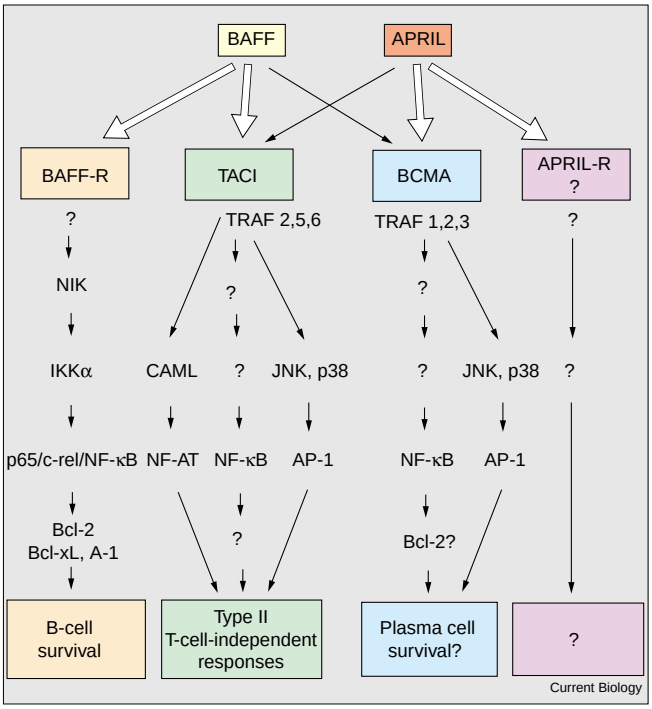
The striking defect in B-cell production in BAFF-deficient mice compared to the (near) normal B lymphopoiesis in TACI-deficient and BCMA-deficient mice may have been explained by functional overlap between these two receptors. However, the recent discoveries by two independent groups (one published in *Current Biology*) of a third BAFF receptor — BAFF-R, also called BR3 — provided a different resolution for this conundrum [5,6]. Like TACI, BAFF-R is a type III transmembrane protein. The extracellular ligand-binding domain of BAFF-R has only a single cysteine-rich domain which contains only four rather than the characteristic six cysteine residues. Although this makes BAFF-R a very distant member of the TNF receptor family, it is anticipated that its three-dimensional structure will be similar to that of its relatives. Remarkably, both groups [5,6] discovered BAFF-R by expression library screening using BAFF as bait, but failed to discover the gene in database searches using profiles specific for TNF-receptors. This demonstrates that functional screens for gene discovery can sometimes be superior to database mining, even after completion of the human and mouse genome projects.

BAFF-R expression was detected in resting B cells [5,6], consistent with a role for BAFF–BAFF-R signaling in the transition from the immature to the mature B cell stage. Injection of soluble BAFF-R proteins into mice diminished IgG1 antibody production [6]. This may indicate that BAFF–BAFF-R signaling is critical for the initiation of B-cell activation whereas CD40L–CD40 stimulation is essential to sustain activation. Since BAFF-R binds only to BAFF and not to APRIL [5,6], this demonstrates that BAFF alone and not APRIL plays a role in B-cell activation. So, do APRIL and BCMA have any role in B lymphopoiesis? BCMA is most highly expressed in Ig-secreting plasma cells [1]. It is therefore tempting to speculate that APRIL–BCMA signaling is critical for survival of those plasma cells in the bone marrow that contribute to long-term memory to infection or immunization.

Genetic and phenotypic analysis of a spontaneous mutant mouse strain (A/WySnJ) with defective production and survival of B cells, demonstrated that BAFF-R is the critical receptor for BAFF during B lymphopoiesis [5–7]. A/WySnJ mice have an insertion in *bcmd*, the gene encoding BAFF-R, and this results in expression of a mutant BAFF-R lacking the last eight amino acids in its intracellular region but retaining normal binding to BAFF [5–7]. This missing region has striking similarity to a corresponding region in BCMA, indicating that these two receptors may activate similar signaling pathways.

As the A/WySnJ mutant BAFF-R is expressed on the cell surface and does bind BAFF, it could potentially act as a dominant-interfering mutant that blocks not only BAFF–BAFF-R signaling but also impedes signals from other (related) receptors. If this were the case, the phenotype of A/WySnJ mutant mice might be more severe than

Figure 1



BAFF preferentially binds to BAFF-R and TACI (thick arrows) and interacts with BCMA more weakly (thin arrow). In contrast, APRIL binds more avidly to BCMA than to TACI and does not bind to BAFF-R. We speculate that a third APRIL receptor exists and expect it to be expressed on epithelial cells. Direct evidence has been presented regarding the signaling cascades that can be activated by TACI and BCMA [1]. So far, nothing has been published on the signaling pathways activated by BAFF-R, but genetic evidence points towards NIK- and IKKα-mediated NF-κB activation which causes upregulation of anti-apoptotic Bcl-2 family members. Sequence homology within the intracellular domains of BAFF-R and BCMA indicate that these two receptors activate similar signaling pathways (although the outcome of BCMA signaling is less clear). TRAF, TNF receptor-associated factor; JNK, c-Jun N-terminal kinase; NF-AT, nuclear factor of activated T cells; CAML, calcium modulator and cyclophilin ligand.

that of BAFF-deficient mice. In fact, the opposite is the case — A/WySnJ mice produce more B cells than BAFF-deficient mice [5–7,11], indicating that the *baff-r* mutation in A/WySnJ mice represents a partial loss-of-function mutation that retains some signaling function.

So, how does BAFF-R signal? It is noteworthy that the effects of BAFF overexpression — prolonged survival and accumulation of B cells, and autoimmunity [8–10] — mimic those observed in transgenic mice overexpressing the pro-survival protein Bcl-2 [19] or those lacking its antagonist Bim [20]. Conversely, the B-cell phenotype of BAFF-deficient and *baff-r* mutant mice is very similar to that caused by Bcl-2 deficiency [21], loss of the NF-κB-inducing kinase NIK in *aly*-mice [22] or the IκB kinase IKKα [23,24], or combined loss of the Rel/NF-κB transcription factors c-Rel and RelA [25]. Reduced levels of Bcl-2 were found in c-Rel/RelA-deficient B cells and expression of a *bcl-2* transgene almost completely rescued the B-cell defect caused by loss of IKKα [24] or c-Rel/RelA [25]. We therefore speculate that BAFF-R promotes B-cell survival by upregulation of Bcl-2 expression mediated by Rel/NF-κB transcription factors (Figure 1): it will be interesting to see if a *bcl-2* transgene or loss of Bim can restore normal B-cell production in BAFF-deficient or BAFF-R-deficient (A/WySnJ) mice.

The importance of BAFF and BAFF-R in controlling the transition of B cells through the B-cell receptor checkpoint makes one wonder whether other members of the TNF family and their receptors might regulate survival and differentiation of T lymphocytes at the transition from the immature $CD4^+CD8^+$ to the mature ($CD4^+CD8^-$ or $CD4^-CD8^+$) stages. Several other questions are raised by these interesting discoveries. For example, what are the functions of APRIL and BCMA? It is unlikely that APRIL has evolved to promote tumorigenesis, but because it stimulates growth of epithelial cancers [12,15] their normal counterparts may be the physiological targets of APRIL. Apparently, these cells express neither BCMA nor TACI [15], indicating that APRIL might have a third receptor, perhaps similar in structure to BAFF-R. The generation of mice lacking APRIL or all of its receptors is expected to unravel the mystery of its physiological function.

Certainly, the demonstration that soluble decoy receptors for BAFF and APRIL can inhibit SLE-like autoimmunity [9] and rheumatoid arthritis [11,26] in susceptible mouse strains and reduce growth of human cancer cell xenografts [15] will further accelerate the race for drugs that inhibit the function of these intriguing molecules.

References

1. Laâbi Y, Strasser A: Lymphocyte survival — ignorance is BLys. *Science* 2000, 289:883-885.
2. Schneider P, MacKay F, Steiner V, Hofmann K, Bodmer J-L, Holler N, Ambrose C, Lawton P, Bixler S, Acha-Orbea H, *et al.*: BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J Exp Med* 1999, 189:1747-1756.
3. Moore PA, Belvedere O, Orr A, Pieri K, LaFleur DW, Feng P, Soppet D, Charters M, Gentz R, Parmelee D, *et al.*: BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science* 1999, 285:260-263.
4. Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, Frew E, Scott ML: An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science* 2001, 293:2111-2114.
5. Thompson JS, Bixler SA, Qian F, Vora K, Scott ML, Cachero TG, Hession C, Schneider P, Sizing ID, Mullen C, *et al.*: BAFF-R, a novel TNF receptor that specifically interacts with BAFF. *Science* 2001, 293:2108-2111.
6. Yan M, Brady JR, Chan B, Lee WP, Hsu B, Harless S, Cancro M, Grewal IS, Dixit VM: Identification of a novel receptor for B lymphocyte stimulator (BLyS) that is mutated in a mouse strain with severe B-cell deficiency. *Curr Biol* 2001, 11:1547-1552.
7. Harless SM, Lentz VM, Sah AP, Hsu BL, Clise-Dwyer K, Hilbert DM, Hayes CE, Cancro MP: Competition for BLyS-mediated signaling through Bcmd/Br3 regulates peripheral B lymphocyte numbers. *Curr Biol* 2001, 11:this issue.

8. Mackay F, Woodcock SA, Lawton P, Ambrose C, Baetscher M, Schneider P, Tschopp J, Browning JL: Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J Exp Med* 1999, 190:1697-1710.
9. Gross JA, Johnston J, Mudri S, Enselman R, Dillon SR, Madden K, Xu W, Parrish-Novak J, Foster D, Lofton-Day C, *et al.*: TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature* 2000, 404:995-999.
10. Khare SD, Sarosi I, Xia XZ, McCabe S, Miner K, Solovyev I, Hawkins N, Kelley M, Chang D, Van G, *et al.*: Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. *Proc Natl Acad Sci USA* 2000, 97:3370-3375.
11. Gross JA, Dillon SR, Mudri S, Johnston J, Littau A, Roque R, Rixon M, Schou O, Foley KP, Haugen H, *et al.*: TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease: impaired B cell maturation in mice lacking BLys. *Immunity* 2001, 15:289-302.
12. Hahne M, Kataoka T, Schröter M, Hofmann K, Irmler M, Bodmer J-L, Schneider P, Bornand T, Holler N, French LE, *et al.*: APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth. *J Exp Med* 1998, 188:1185-1190.
13. Marsters SA, Yan M, Pitti RM, Haas PE, Dixit VM, Ashkenazi A: Interaction of the TNF homologues BLyS and APRIL with the TNF receptor homologues BCMA and TACI. *Curr Biol* 2000, 10:785-788.
14. Thompson JS, Schneider P, Kalled SL, Wang L, Lefevre EA, Cachero TG, MacKay F, Bixler SA, Zafari M, Liu Z-Y, *et al.*: BAFF binds to the tumor necrosis factor receptor-like molecule B cell maturation antigen and is important for maintaining the peripheral B cell population. *J Exp Med* 2000, 192:129-136.
15. Rennert P, Schneider P, Cachero TG, Thompson J, Trabach L, Hertig S, Holler N, Qian F, Mullen C, Strauch K, *et al.*: A soluble form of B cell maturation antigen, a receptor for the tumor necrosis factor family member APRIL, inhibits tumor cell growth. *J Exp Med* 2000, 192:1677-1684.
16. Xu S, Lam KP: B-cell maturation protein, which binds the tumor necrosis factor family members BAFF and APRIL, is dispensable for humoral immune responses. *Mol Cell Biol* 2001, 21:4067-4074.
17. von Bülow G, van Deursen JM, Bram RJ: Regulation of the T-independent humoral response by TACI. *Immunity* 2001, 14:573-582.
18. Yan M, Wang H, Chan B, Roose-Girma M, Erickson S, Baker T, Tumas D, Grewal IS, Dixit VM: Activation and accumulation of B cells in TACI-deficient mice. *Nat Immunol* 2001, 2:638-643.
19. Strasser A, Whittingham S, Vaux DL, Bath ML, Adams JM, Cory S, Harris AW: Enforced ***BCL2*** expression in B-lymphoid cells prolongs antibody responses and elicits autoimmune disease. *Proc Natl Acad Sci USA* 1991, 88:8661-8665.
20. Bouillet P, Metcalf D, Huang DCS, Tarlinton DM, Kay TWH, Köntgen F, Adams JM, Strasser A: Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. *Science* 1999, 286:1735-1738.
21. Veis DJ, Sorenson CM, Shutter JR, Korsmeyer SJ: Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. *Cell* 1993, 75:229-240.
22. Shinkura R, Kitada K, Matsuda F, Tashiro K, Ikuta K, Suzuki M, Kogishi K, Serikawa T, Honjo T: Alymphoplasia is caused by a point mutation in the mouse gene encoding Nf-κb-inducing kinase. *Nat Genet* 1999, 22:74-77.
23. Senftleben U, Cao Y, Xiao G, Greten FR, Krähn G, Bonizzi G, Chen Y, Hu Y, Fong A, Sun SC, Karin M: Activation by IKKα of a second, evolutionary conserved, NF-κ B signaling pathway. *Science* 2001, 293:1495-1499.
24. Kaisho T, Takeda K, Tsujimura T, Kawai T, Nomura F, Terada N, Akira S: IκB kinase α is essential for mature B cell development and function. *J Exp Med* 2001, 193:417-426.
25. Grossmann M, O'Reilly LA, Gugasyan R, Strasser A, Adams JM, Gerondakis S: The anti-apoptotic activities of rel and RelA required during B-cell maturation involve the regulation of Bcl-2 expression. *EMBO J* 2000, 19:6351-6360.
26. Wang H, Marsters SA, Baker T, Chan B, Lee WP, Fu L, Tumas D, Yan M, Dixit VM, Ashkenazi A, Grewal IS: TACI-ligand interactions are required for T cell activation and collagen-induced arthritis in mice. *Nat Immunol* 2001, 2:632-637.